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Formation of 2n gametes in durum wheat haploids: Sexual polyploidization

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Summary

Allopolyploidy, resulting from interspecific and intergeneric hybridization accompanied by sexual doubling of chromosomes, has played a major role in the evolution of crop plants that sustain humankind. The allopolyploid species, including durum wheat, bread wheat, and oat, have developed a genetic control of chromosome pairing that confers on them meiotic regularity (diploid-like chromosome pairing), and hence reproductive stability, and disomic inheritance. Being natural hybrids, they enjoy the benefits of hybridity as well as polyploidy that make them highly adaptable to diverse environments. Despite the complexities of sexual reproduction, it is widespread among plants and animals. Sexual polyploids are highly successful in nature. Sexual polyploidization is far more efficient than somatic chromosome doubling. Sexual polyploidization effected by functioning of unreduced ($2n$) gametes in the parental species or in their hybrids has been instrumental in producing our grain, fiber, and oilseed crops. Evidence is presented for the occurrence of sexual polyploidization in durum haploids. The *Ph1*-induced failure of homoeologous pairing is an important factor in the formation of first division restitution (FDR) nuclei and $2n$ gametes. The evolutionary and breeding significance of sexual polyploidization is discussed. It is emphasized that three factors, viz., sexual reproduction, allopolyploidy, and genetic control of chromosome pairing, jointly constitute a perfect recipe for cataclysmic evolution in nature.

Introduction

Polyploidy has played a significant role in the evolution of plant species useful to man. Allopolyploidy or amphidiploidy, resulting from hybridization coupled with induction of chromosome doubling, has been instrumental in the production of many of the most important grain, forage, and fiber crops. The grass family (Poaceae), in particular, has a preponderance of allopolyploids (alloploids)[1]. The allopolyploid species are highly adaptable to adverse environments and hence are widely distributed. Being natural hybrids, they enjoy the benefits of both hybridity and polyploidy. The enzyme diversity coded by related genes in different genomes in allopolyploids may contribute to their selective advantage and fitness (Adams & Allard, 1977; see also Jauhar, 1993, Chapter 8).

Several factors contribute to the preponderance of allopolyploids in the grasses (Stebbins, 1956; Jauhar & Singh, 1969; see also Jauhar, 1981, Chapter 7). These include: (1) sympatric distribution of grass species giving ample opportunity for hybridization; (2) abundance of wind-borne pollen and occurrence of self-incompatibility systems, both promoting cross hybridization; (3) perennial growth habit coupled with efficient means of vegetative propagation facilitating

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[1] The terms 'allopolyploid' and 'alloploid' are used interchangeably in this article; 'autopolyploid' and 'autoploids' are also used interchangeably.

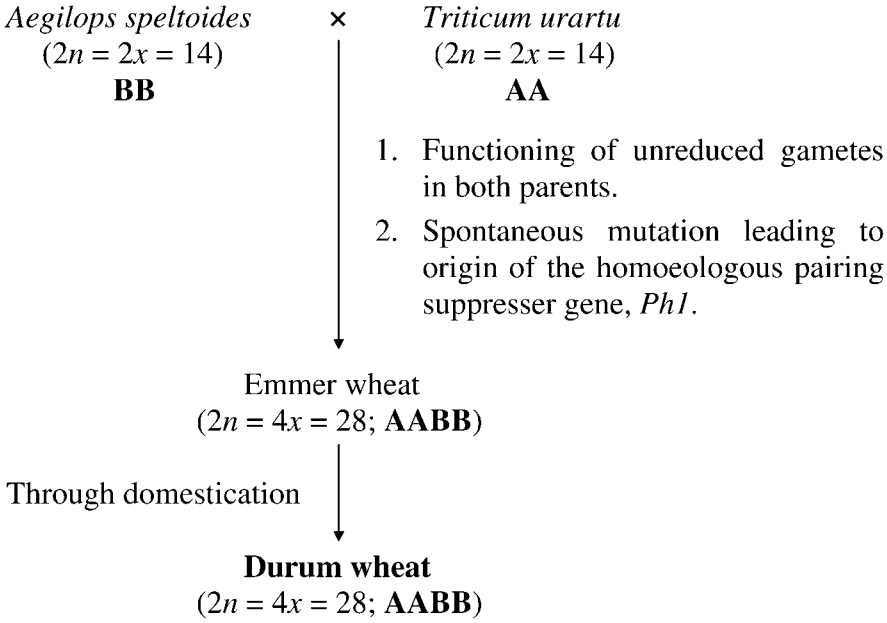

Figure 1. Steps in the evolution of durum wheat.

the survival of grass species and their hybrids for extended periods; (4) natural decapitation of hybrids (e.g., severe grazing by cattle or wild animals) converting them into amphidiploids. Although spontaneous somatic chromosome doubling induced, for example, by natural decapitation of natural or synthetic hybrids would lead to production of amphidiploids (Jauhar & Singh, 1969), meiotic non-reduction (functioning of unreduced female and male gametes) plays a more significant role in producing allopolyploids in nature (Harlan & de Wet, 1975; de Wet, 1980).

Durum wheat or macaroni wheat (*Triticum turgidum* L.) is an important cereal used for human consumption worldwide. It is grown in several European countries, including Italy, France, Turkey, Romania, and Ukraine, and in Canada. And it is an important wheat of the Northern Great Plains of the United States, the state of North Dakota being the number one durum producer. By hybridizing with maize (*Zea mays* L.) followed by chromosome elimination, we produced haploids in several commercial durum cultivars grown in North Dakota (Almouslem et al., 1968). During meiosis, many of these haploid plants formed unreduced ($2n$) gametes, mainly by first division restitution (FDR) which produced viable seeds and thereby disomic tetraploid durum plants. This demonstrates the role of $2n$ gametes in rapid polyploid production. And this phenomenon may be widespread in nature. Meiotic non-reduction in durum haploids is described in detail and its role in sexual polyploidization discussed in this article.

Definitions and terminology

It would be desirable to define various terms to be used in this article. Haploid plants are sporophytes with half the normal chromosome complement. A plant with half the chromosome number, derived from a diploid species, is called a *monoploid, monohaploid*, or simply a *haploid*. Monoploids have only one set of chromosomes but because they are sporophytes, their somatic chromosome number is denoted by $2n$. While $2n$ refers to the somatic chromosome number, x indicates the basic chromosome number, i.e., the number of chromosomes in one genome. A monoploid would therefore have only one genome in a single dose. A haploid or monoploid derived from diploid Einkorn wheat (*Triticum monococcum* L.) or from diploid barley (*Hordeum vulgare* L.), for example, is indicated to have $2n = x = 7$ chromosomes. On the other hand, a haploid derived from a polyploid species like hexaploid bread wheat (*Triticum aestivum* L., $2n = 6x = 42$) or tetraploid durum wheat ($2n = 4x = 28$) is preferably termed a *polyhaploid*. Thus, a polyhaploid of durum wheat has $2n = 2x = 14$ chromosomes.

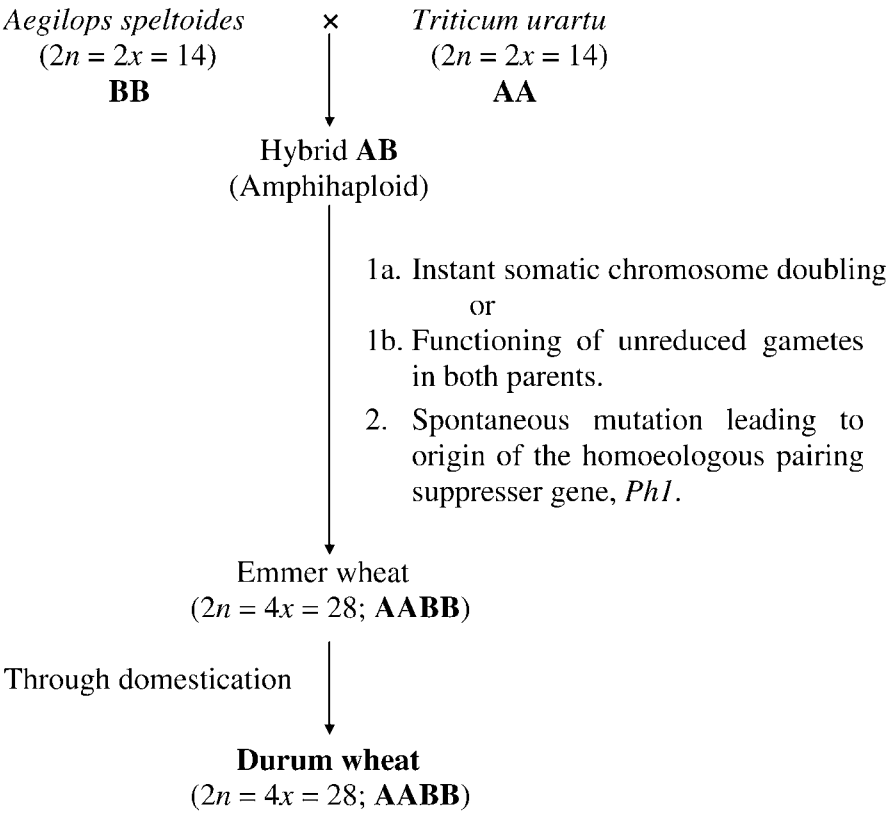

Figure 2. Steps in the evolution of durum wheat via instant somatic chromosome doubling or functioning of 2*n* male and female gametes in the AB hybrid (amphihaploid).

A polyhaploid, in turn, is further defined according to the nature of polyploidy of the parental species from which it is derived. A polyhaploid from an allopolyploid like durum wheat is termed as an *allopolyhaploid* (2*n* = 2*x* = 14), whereas one derived from an autopolyploid like the potato (*Solanum tuberosum* L., 2*n* = 4*x* = 48) is described as an *autopolyhaploid*. However, the term 'haploid' is widely used in the case of both diploid and polyploid species.

Cytogenetic architecture of durum wheat

For producing durum haploids and studying their sexual polyploidization, it would be helpful to understand durum wheat's genomic constitution. Durum wheat is a true breeding natural hybrid. It is an allotetraploid with the genomic constitution of AABB. Its two genomes were derived from two diploid wild species. The donor of the A genome is *Triticum urartu* Tumanian (Nishikawa, 1983; Dvořák et al., 1993), a species closely related to *Triticum monococcum* L., and the available evidence shows that the B genome most probably was derived from *Aegilops speltoides* Tausch. (Wang et al., 1997; Dvořák, 1998; see also Jauhar, 1996). The two progenitors, which are native to the Middle East, hybridized in nature some 500,000 years ago (Huang et al., 2002) and gave rise to tetraploid emmer wheat (*Triticum turgidum* var. *dicoccoides* Körn), presumably in one step as a result of functioning of unreduced gametes in both parents (Figure 1). This is a plausible explanation because the sterile, presumably annual, hybrid AB or amphihaploid, if ever formed, would probably not have survived in nature very long. However, if the hybrid AB was indeed produced, it could have undergone instant somatic chromosome doubling of the type described by Jauhar and Singh (1969) or, more plausibly, meiotic doubling by FDR as discussed in Jauhar et al. (2000; Figure 2). The wild emmer through domestication gave rise to cultivated durum wheat.

The corresponding chromosomes of the two genomes of durum wheat are closely related, comprising seven homoeologous groups of two chromosomes each. Genetically and evolutionarily related chromosomes are called *homoeologs*. Because the homoeologous chromosomes (e.g., chromosome 1 of the A genome and chromosome 1 of the B genome) are closely related and hence capable of pairing with one another, a genetic control on pairing would be necessary. Therefore, at the time of origin of tetraploid emmer wheat a spontaneous mutation must have given rise to the homoeologous chromosome pairing suppresser gene, *Ph1*, which conferred meiotic regularity and hence reproductive stability to the newly evolved wheat. The complete absence of *Ph1,* in 5D(5B) substitution haploids (Jauhar et al., 1999) or in *ph1c*-haploids (Almouslem et al., 1998) obtained from Cappelli durum mutant (Giorgi, 1978), leads to extensive homoeologous pairing. The origin of *Ph1* would have been essential for reproductive stability and survival of polyploid wheats.

Production of haploids: Their importance

The usefulness of haploid plants in basic research in cytogenetics as well as in practical plant breeding has been well documented (Jain et al., 1996). They can be usefully employed in studies on induced mutagenesis, gene dosage effects, and linkage analyses. Using haploid tissue cultures, with only one allele of each gene, the efficacy of different mutagens can be studied more effectively. Haploids also offer an opportunity for studying chromosome pairing relationships because the intergenomic (and perhaps intragenomic) homologies, that generally remain masked in the parental species, are revealed in the complement in the haploid state. Thus, the study of chromosome pairing in polyhaploids of cultivated wheats, both bread wheat (Jauhar et al., 1991) and durum wheat (Jauhar et al., 1996), has helped in studying intergenomic relationships and genetic control of chromosome pairing. Haploids have also been usefully employed in practical breeding. Haploid-derived homozygous lines provide a rapid means of achieving homozygosity, thereby accelerating plant breeding programs (Baenziger, 1996). It is remarkable that as early as 1924, Blakeslee and Belling envisioned the use of haploids 'as a new and simple way to establish pure lines from highly heterozygous plants.'

Several methods of extracting haploids have been tried in durum wheat. These include anther culture (Ghaemi et al., 1993; Doğramacı-Altuntepe et al., 2001), ovary culture (Sibi et al., 2001), and chromosome elimination after hybridization with maize, *Zea mays* L. (Sarrafi et al., 1994; Almouslem et al., 1998). Several researchers have shown that maize-induced haploid production is the method of choice for both bread wheat (Laurie et al., 1990) and durum wheat (O'Donoughue & Bennett, 1994; Almouslem et al., 1998; Knox et al., 2000; Jauhar, 2002). Using the maize technique, we produced haploids on a large scale in seven commercial durum cultivars grown in North Dakota (Almouslem et al., 1998). And it is the study of these haploids that has contributed to an understanding of sexual polyploidization described in this article.

Chromosome techniques

Several methods of staining chromosomes are known: acetocarmine, acetoorcein, Feulgen staining, etc (Phillips, 1981). For conventional staining of somatic and meiotic chromosomes, we routinely use carbol fuchsin stain (L.R. Joppa, pers. commun.) according to the technique described earlier (Jauhar et al., 2000; Jauhar, 2002). In somatic spreads, 14 chromosomes of the haploid complement can be counted easily, with one dose each of the satellited chromosomes 1B and 6B (see Figure 3A). In conventionally stained somatic cells, the chromosomes of the A and B genomes cannot be identified. It is, however, important to characterize chromosomes of the two genomes, for which we use fluorescent genomic *in situ* hybridization (fl-GISH). Because we use total genomic DNA along with fluorescent material as a probe in hybridization, we prefer to call this technique 'fl-GISH.' However, the terms 'fl-GISH' and 'GISH' are used interchangeably by some researchers.

Fl-GISH provides an excellent tool for genomic painting and to distinguish the chromosomes of the A- and B genomes (Jauhar et al., 1999) (see Figures 5A-F). The GISH analysis is conducted by hybridizing the A-genome chromosomes with *Triticum urartu* genomic DNA (labeled with biotin-14-dATP) while blocking the B genome with *Aegilops speltoides* genomic DNA. It is significant that the total genomic DNA of *Ae. speltoides* effectively blocks the B genome of durum wheat, lending further support for this grass being the donor of B genome. The chromosome

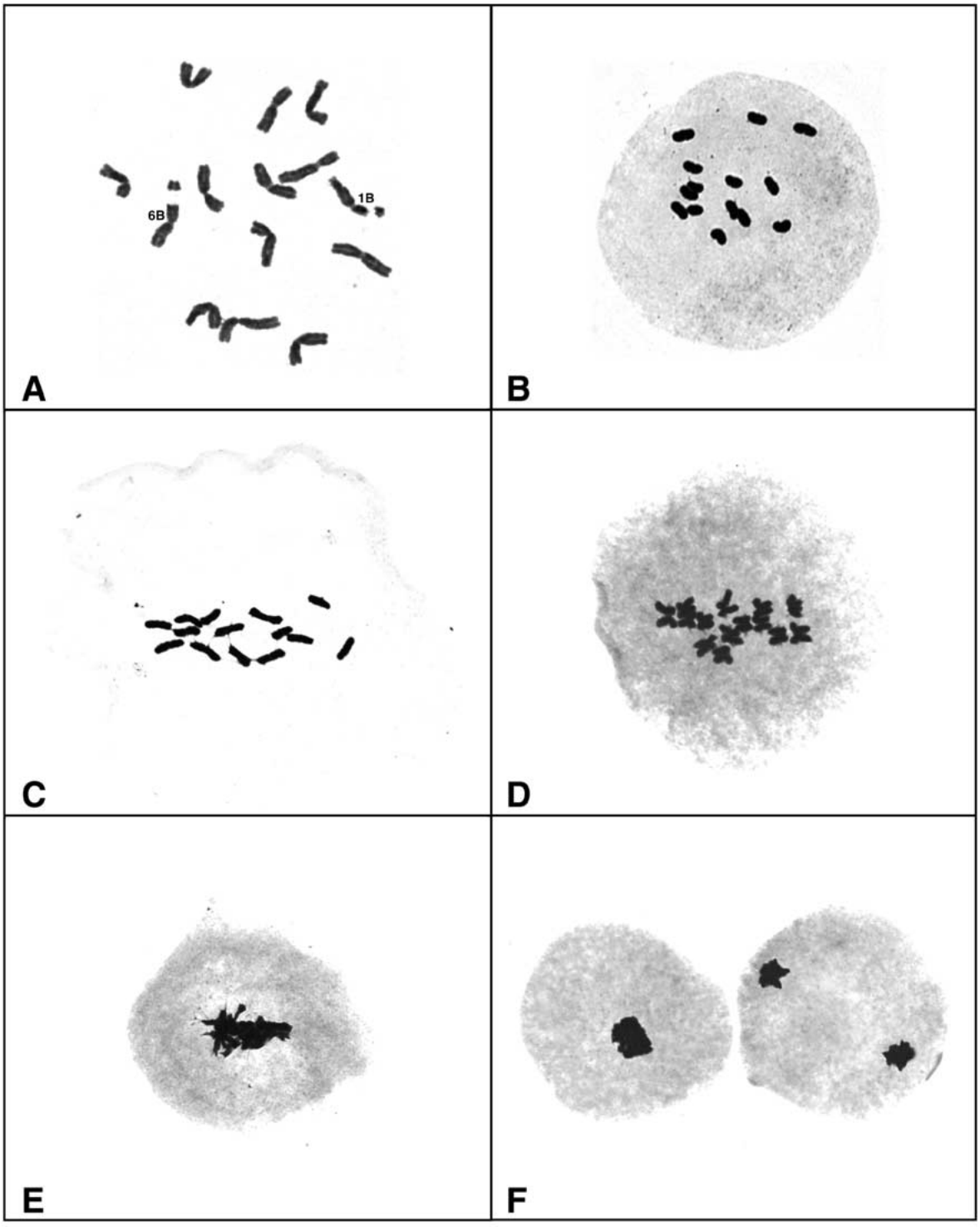

Figure 3. Somatic and meiotic chromosomes of durum haploids with *Ph1*. **A.** 14 somatic chromosomes from root-tip cells. Note two satellited chromosomes 1B and 6B **B.** A pollen mother cell (PMC) with 14 univalents (lack of homoeologous pairing because of *Ph1*. **C.** The 14 univalents aligned on metaphase plate. **D.** All 14 univalents split into chromatids at meta-anaphase I, while arranged on the metaphase plate. **E.** All divided univalents fail to move to poles and clump in the center, resulting in first division restitution (FDR). **F.** An undivided FDR (left) and a divided (equational division) FDR nucleus resulting in a dyad (right).

preparations are counterstained with propidium iodide (PI) and the labeled DNA is detected using fluorescein isothiocyanate (FITC).

Meiotic non-reduction and seed set on *Ph1*-haploids

Using the maize technique, Jauhar and coworkers produced a total of 101 haploid plants in seven commercial durum cultivars with the *Ph1* gene that suppresses homoeologous pairing (Almouslem et al., 1998). As expected, these *Ph1*-haploids formed mostly 14 univalents at metaphase I of meiosis (Figures 3B & C), and consequently showed abnormalities at anaphase I through telophase and cytokinesis (Figures 3D-F). However, despite the meiotic abnormalities, the *Ph1*-haploids produced some seed albeit in low frequency (Table 1).

In detailed studies on pollen mother cells (PMCs), we observed that two mechanisms resulted in the formation of 2*n* gametes, which in turn led to viable seed set, and disomic durum plants (Jauhar et al., 2000).

First division restitution (FDR)

At meiotic metaphase I, the 14 univalents in the *Ph1*-haploids organized themselves on the equatorial plate (Figure 3C) and, instead of moving to the poles, they split into chromatids (Figure 3D). All divided univalents clumped at the plate and resulted in restitution nuclei (Figure 3E), bypassing the first meiotic division (the reductional division) and thus having the full complement of 14 chromosomes. The restitution nuclei sometimes underwent a normal second (equational) division and formed dyads (Figure 3F) instead of tetrads. The dyads in turn formed functional unreduced male gametes with 14 chromosomes. It is likely that on the female side the same mechanism led to the formation of unreduced gametes, which on fusion with the unreduced male gametes formed the viable seed that we observed.

Genetic control of chromosome pairing: A prerequisite for meiotic non-reduction?

FDR plays an important role in the production of unreduced gametes. Our observations on durum haploids, although somewhat limited, show that *Ph1*-induced failure of homoeologous chromosome pairing (occurrence of univalents) at meiosis I may be important, if not a prerequisite, for the occurrence of FDR (Jauhar et al., 2000). This phenomenon occurred only in *Ph1*-haploids that formed mostly 14 univalents that tended to align themselves on the plate (Figure 3C), a condition apparently necessary for the splitting of univalents (Figure 3D) and formation of FDR (Figure 3E). However, it was not observed in 5D(5B) substitution haploids that lacked *Ph1* and hence showed extensive chromosome pairing (Jauhar et al., 2000; Doğramacı-Altuntepe & Jauhar, 2001) because the paired chromosomes tend to segregate to the poles. Xu & Joppa (2000) also observed a reduction or even absence of FDR formation in hybrids of 5D(5B) substitution with *Aegilops squarrosa*.

Unequal movement of chromosomes (univalents) at meiotic anaphase I

The 14 univalents in the *Ph1*-haploids sometimes showed equal (7:7) distribution to the poles (Figure 4A). The identity of the chromosomes thus distributed was difficult to ascertain in conventionally stained PMCs. However, FISH on such PMCs showed random movement of the A-genome and B-genome chromosomes. Thus, Figure 5A shows a 7:7 distribution, which, on fluorescent GISH analysis, revealed 2:5 distribution of the A-genome chromosomes and 5:2 distribution of the B-genome chromosomes (Figure 5B).

As a result of random movement of univalents at anaphase I, all possible distributions, i.e., 7:7, 8:6, 9:5, 10:4, 11:3, 12:2, and 13:1 were observed (Figures 4A-G). In extreme cases, all chromosomes moved to one pole (Figure 4H) and formed a nucleus with 14 chromosomes, resulting essentially in meiotic non-reduction (Figure 4 I) witnessed in FDR described above. These nuclei, that effectively bypassed the first (reductional) division, underwent the second (equational) division and produced unreduced gametes with 14 chromosomes.

Characterization of disomic nature of seed-derived plants: Evolutionary implications

We believe that the two mechanisms of meiotic non-reduction described above gave rise to viable seed on haploid durum plantlets. The seeds were germinated and gave rise to normal plantlets with a typical phenotype of the parental cultivar from which they were derived. Somatic cells from root-tips of seed-derived plantlets demonstrated their tetraploid status with 2*n* =

Table 1. Seedset on haploid plants of seven durum cultivars

Cultivar	Total no. euhaploids	No. euhaploid plants with set seed	No. of seeds set	Mean no. seed / plant	SE of the mean
Cappelli	2	1	2	1.00	1.00
Durox	25	3	22	0.88	0.80
Langdon	4	4	11	2.75	1.44
Lloyd	28	2	38	1.36	1.01
Medora	23	3	12	0.52	0.33
Monroe	3	2	4	1.33	0.88
Renville	16	3	10	0.63	0.45
Total	101	18	99	0.98	0.36

From Jauhar et al. (2000).

28 chromosomes (Figure 5C). Fl-GISH analysis confirmed the disomic nature of the plantlets, with 14 brightly lit A-genome chromosomes and 14 B-genome chromosomes faded in the background (Figure 5D). A double dose of the evolutionary translocation 4A·7B was also discernible in disomic cells (Figure 5D), testifying to the disomic status of the meiotically doubled plants.

Meiosis in the seed-derived plants further confirmed their disomic status. Seven bivalents of the A genome and 7 of the B genome (Figures 5E & F) demonstrated precise duplication of the durum chromosome complement. Clearly, meiotic non-reduction gave rise to sexual polyploidization, a mechanism that may have played an important role in producing a preponderance of allopolyploids in nature.

Sexual polyploidization: An important evolutionary force

There is evidence that allopolyploids arise in nature primarily through sexual polyploidization or meiotic non-reduction (Harlan & de Wet, 1975; see Jauhar, 1981, Chapter 11). Unreduced gametes of both parents fuse to give rise to fertile amphidiploids, which benefit from hybridity and polyploidy and may succeed as new species. The union of two or more genomes from different species confers an opportunity for new, useful gene interactions that are missing in parental diploids or derived autotetraploids. Many of the crop plants that feed the world are of allopolyploid origin and arose via sexual polyploidization in one or sometimes two steps. Thus, durum wheat probably evolved in one step as an amphidiploid between two wild grasses, *Ae. speltoides* (BB) and *T. urartu* (AA), perhaps about half a million years ago (Figure 1). Alternatively, unreduced male and female gametes in the AB hybrid (amphihaploid) probably functioned to give rise to amphidiploid AABB (see Jauhar et al., 1999, 2000; Figure 2). The hexaploid wheat (AABBDD) evolved later, about 8,000 years ago (Huang et al., 2002), via natural hybridization of tetraploid emmer wheat, cultivated or wild, with a third diploid wild grass *Ae. tauschii* Coss. that contributed the D genome (McFadden & Sears, 1946; Dvořák et al., 1998). Again, unreduced gametes in both parents or in the triploid hybrid ABD would have given rise to bread wheat whose meiosis was regularized by the *Ph1* that must have originated earlier in tetraploid wheat (see Figure 1).

That sexual polyploidization is an important evolutionary force is evidenced by spontaneous production of amphidiploids in the tribe Triticeae (Maan & Sasakuma, 1977; Stefani, 1986; Xu & Joppa, 1995, 2000; Cox, 1998), the *Festuca-Lolium* complex (Jauhar, 1993), and several other plant groups of monocots and dicots, e.g., the Brassiceae (Heyn, 1977) and *Solanum* species (Watanabe & Peloquin, 1989).

Sexual polyploidization: Breeding value

Unreduced gametes are of importance for basic studies in cytogenetics as well as applied plant breeding and facilitate the production of new polyploid species (e.g., Figures 1 and 2). Sexual polyploidization is clearly of breeding value in that fertile amphidiploids can be produced in one step, obviating the need for cumbersome colchicine treatments of sterile F_1 hybrids. Fertile amphidiploids can be used for introducing alien genes into crop plants. Thus, in the

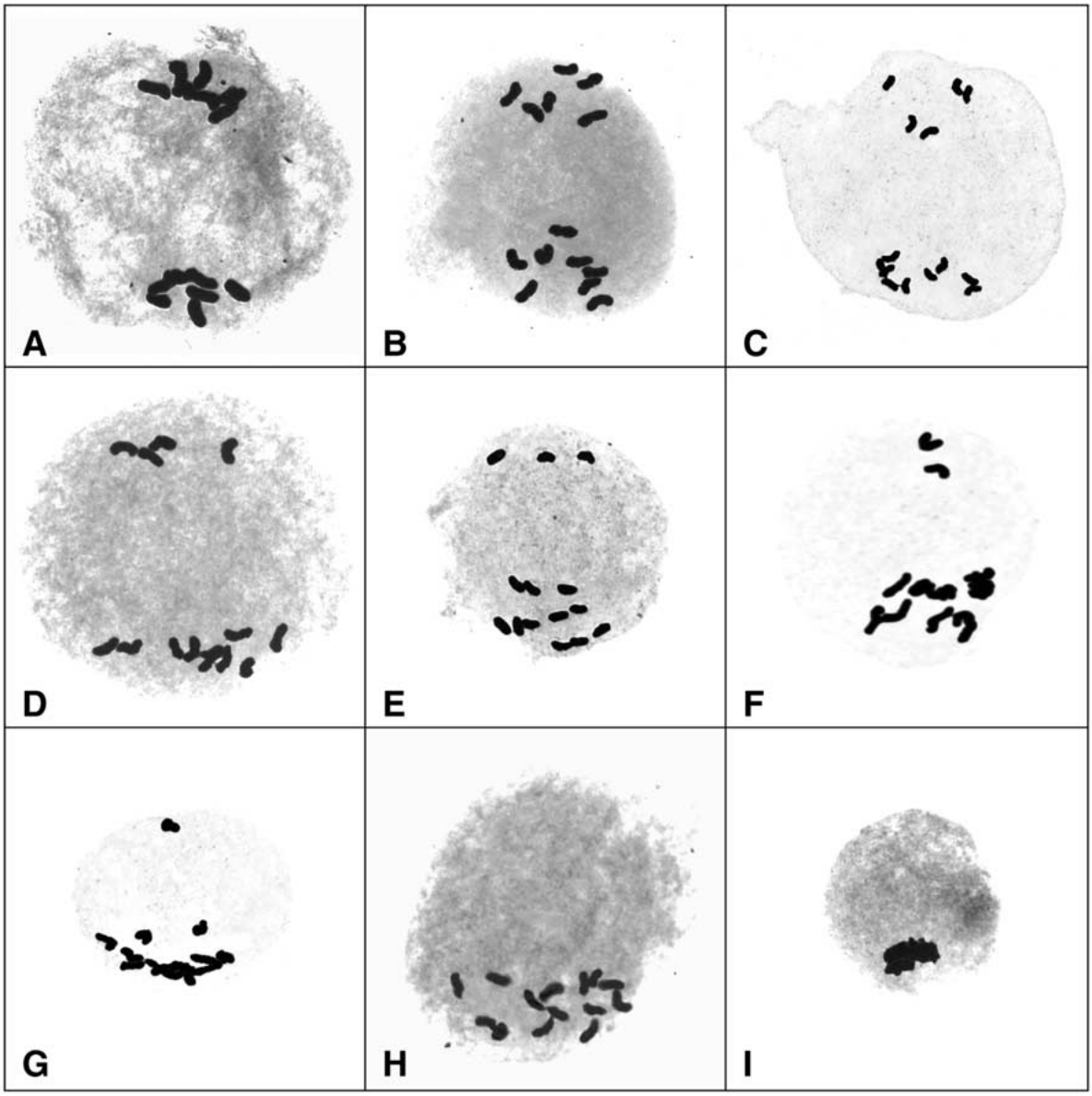

Figure 4. Unequal distribution of univalents at anaphases in *Ph1*-haploids of durum wheat. **A.** 7:7 separation. **B**. 6:8 separation. **C.** 5:9 separation. **D.** 4:10 separation. **E.** 3:11 separation. **F**. 2:12 separation. **G.** 1:13 separation**. H.** 0:14 separation. **I.** A PMC at a stage equivalent to telophase I with all chromosomes on one pole.

Triticeae, spontaneous amphidiploids provide a valuable genetic resource for introgressing desirable alien genes into cultivated wheats (Cox, 1998). Unreduced gametes also help synthesize triploids of crop plants, e.g., maize (Curtis & Doyle, 1992). Such triploids can in turn help produce aneuploids.

Meiotic mutants producing 2*n* gametes have been usefully employed in exploiting hybrid vigor, and methods of maximizing hybrid vigor in polyploids have been described (Peloquin, 1982; Buso et al., 1999). 2*n* gametes were reported in many tuber-bearing species of *Solanum* (Watanabe & Peloquin, 1989) and the cytological mechanisms of their formation were elucidated clearly in microsporogenesis (Mok & Peloquin, 1975; Ramanna, 1974, 1979, 1983) and in megasporogenesis (Iwanaga & Peloquin, 1979;

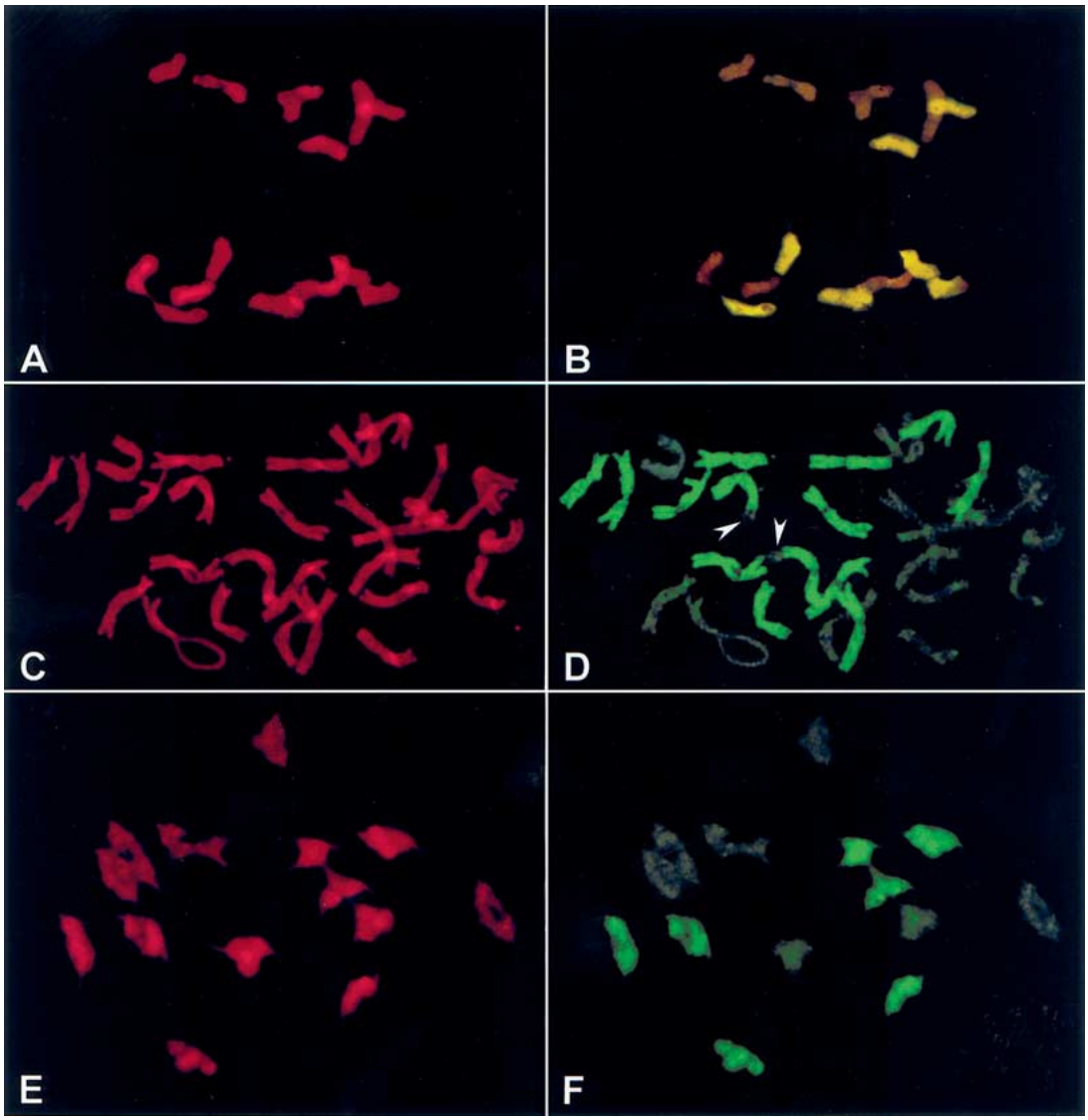

Figure 5. Fluorescent genomic in situ hybridization (GISH) analysis of meiotic chromosomes of durum haploids (A, B); and somatic (C, D) and meiotic (E, F) chromosomes of sexually doubled durum plants. **A.** Ana-telophase I with propidium iodide (PI)-stained univalents, showing 7:7 distribution. **B.** Same cell as in **A** probed with biotinylated A-genome DNA, showing 2:5 distribution of A-genome chromosomes (brightly lit) and 5:2 separation of B-genome chromosomes faded into the background. **C.** 28 somatic chromosomes (counterstained with PI) of seed-derived disomic durum plants. **D.** Same cell as in **C** after hybridization with the A-genome probe, showing 14 brightly lit chromosomes of the A genome. Note 14 B-genome chromosomes faded in the background. **E.** 14 bivalents in seed-derived disomic plants. Note 13 ring and 1 rod bivalents. **F.** Same cell as in **E** after probing with the A-genome DNA, showing 7 fluorescing bivalents of the A genome and 7 faded bivalents of the B genome.

Stelly & Peloquin, 1986). Peloquin (1982) estimated that the 2*n* gametes formed by FDR transmit 80% of the genotype of diploids to their tetraploid progeny in 4*x*-2*x* and 2*x*-4*x* crosses (Peloquin, 1982). Heterosis in the 4*x* progeny was ascribed to the nearly intact transfer of parental heterozygosity and epistatic interactions through FDR gametes. When a tetraploid cultivar is crossed with a diploid FDR genotype, unilateral sexual polyploidization may occur with high frequency and result in highly heterozygous and heterotic tetraploid progeny. From 4*x* × 2*x* crosses in potato, Okwuagwu and Peloquin (1981) obtained 4*x* progeny as a result of 2*n* pollen production by the 2*n*

parent. The 4*x* progeny showed significant heterosis for tuber yield.

Sexuality, polyploidy, and genetic control of chromosome pairing

The sex paradox

Sex has long intrigued poets, philosophers, as well as scientists, particularly cytogeneticists and evolutionists. Evolutionary biologists have had a hard time explaining the existence and evolution of sex. From a practical standpoint it would be far more efficient and convenient to have an asexual mode of reproduction rather than a sexual mode. Thus, a parthenogenetic female animal could reproduce much faster because it would have no need to hunt for a proper mate. Similarly, an apomictic plant species could reproduce much more rapidly than its sexual counterpart because the former would not have to seek viable pollen from an appropriate source at the right time of receptivity. Despite these apparent advantages of asexuality, why has sex originated, survived, and evolved in nature, and why are sexual (amphimictic) species far more predominant than their apomictic counterparts? This has been one of the enduring enigmas of evolutionary biology.

Several theories have been advanced to explain the widespread occurrence of sexual reproduction (Rice, 2002; Otto & Lenormand, 2002). The process of meiosis and consequent genetic recombination, inseparably associated with sexual reproduction, confers the benefit of exchange of genetic material among members of a species. Chromosome pairing during meiosis helps create genetic variability not only by reshuffling full chromosomes but also parts of chromosomes, helping the survival of sexually reproducing organisms in ever changing environments.

Sex and evolution

Meiosis is an essential tool of sexual organisms in bringing about re-assortment of genes and maintaining their chromosome number. Evolution of sex and meiosis must have occurred concurrently because both are necessary for efficient survival and evolution. Sexual reproduction offers a great adaptive advantage in terms of recombination of existing and newly arisen mutant genes and their reshuffling among the progeny to produce novel gene combinations, which confer adaptive advantages for inhabiting new environments.

Mutation provides a major and perhaps the only real source of variation in an asexual organism, although somatic crossing-over may in some cases also contribute to variation (Vig, 1973; Lassner & Orton, 1983; D'Amato, 1990). Most mutations are in fact deleterious. Sex and recombination help remove deleterious mutations, thereby improving fitness of sexually reproducing organisms. If a mutation is useful, it may confer adaptive advantage and be perpetuated in the clonal progeny. However, if two beneficial mutations occur in two different individuals, it would be difficult to combine their benefits into one individual. In this respect, asexual organisms (apomictic plants, for example) are closed systems or evolutionary blind alleys. Plants that reproduce sexually thrive and evolve in diverse environments and therefore are dominant in nature.

Apomixis, sex, and polyploidy: Their interdependence

Polyploids, except the diploidized allopolyploids, tend to be more sterile than their diploid counterparts (Lewis, 1980). To overcome the sterility problem, the polyploids may become apomictic. Apomixis is asexual reproduction through seed, which produce matromorphic progeny facilitating the fixation of maternal genotype. Thus, the maternal heterozygosity if any would be fixed facilitating exploitation of hybrid vigor.

There is a remarkable correlation between polyploidy, particularly aneuploidy, and the apomictic mode of reproduction in the grass family (Nygren, 1954; Grimanelli et al., 1998). For example, *Poa* species with high levels of aneuploidy are apomictic; *P. subcoerulea* has $2n = 28 - 147$ chromosomes (Carnahan & Hill, 1961). Thus, polyploid agamic complexes resort to apomixis – a step necessary to escape from sterility and hence to survive in nature. Apomixis is merely an instrument of survival and hardly plays any role in evolution. Sexual polyploids are much more successful than apomictic ones.

Sex and allopolyploidy: An important linkage

Among the sexual polyploids, allopolyploids are much more successful and hence occur in much greater frequency than autopolyploids (Grant, 1981). Autopolyploidy does not add any new genes and confers little adaptive advantage, although dosage effect of genes may be helpful. Moreover, it is accompanied by high sterility unless there is cytological diploidization (Jauhar, 1970) or some other (genetic) control

that ensures regular disjunction of multivalents (Crowley & Rees, 1968). Evolution by straight chromosome multiplication is therefore slow and inefficient, and hence autoploids are of rather rare occurrence in nature. Sexuality combined with allopolyploidy is an important force in evolution. Because allopolyploidy involves interspecific or intergeneric hybridization associated with chromosome doubling, alloploidy occurs only in sexually reproducing organisms – a linkage that promotes evolution.

Sexuality, allopolyploidy, and genetic control of chromosome pairing: A perfect recipe for rapid evolution

Mutations have played an important role in bringing about diversity in the biological kingdom and hence are a force in evolution. However, because natural mutations occur in low frequency, the process of evolution and speciation is of necessity very slow. Therefore, as early as 1792, Blake was led to state: 'To create a little flower is the labour of ages.' A little flower in this quotation means a new species.

The catalytic effect of hybridization on evolution has been known for a long time. Hutchinson (1953) stated that the most important phase in the evolution of most cultivated plants is natural hybridization followed, or perhaps accompanied, by natural polyploidization that could very well occur by functioning of 2*n* gametes in the parents (Harlan & de Wet, 1975; Figure 1) or in their hybrid or amphihaploid (Jauhar et al., 2000; Figures 3 & 4). Many of our important food (e.g., durum wheat, bread wheat, and oats), fiber (cotton), 'luxury' (tobacco), oilseed (mustard, *Brassica* spp.), and fodder (e.g., tall fescue and Napier grass) crops owe their origin to this powerful evolutionary mechanism.

The catalytic effect of hybridization on evolution lies primarily in enlarging the size of the gene pool, which would facilitate a favorable response of a population to a changing environment (Grant, 1963; Stebbins, 1969, 1974). Superimposed upon this effect is a higher mutation frequency in hybrids than in the parental species (Stebbins, 1969), which could further accelerate the pace of evolution.

The man-made cereal, triticale – a hybrid between *Triticum* species and *Secale cereale* L., provides an excellent example of accelerated evolution through alloploidy. Such a shortcut to speciation has been referred to as saltational speciation (Lewis, 1966), cataclysmic evolution, or even evolution by large quantum jumps (see Jauhar, 1975d). This fact led Haldane (1958) to modify Blake's 1792 famous dictum to read: 'To create a little flower is the labour of ages, *except by allopolyploidy.*' (The italicized portion was added by Haldane.)

Since then we have also known that the successful establishment of a sexually reproducing allopolyploid depends upon the integration of constituent genomes into a meiotically, and hence reproductively, stable form that can be effectively achieved by a precise genetic regulation of chromosome pairing of the type demonstrated in hexaploid bread wheat (Riley & Chapman, 1958; Sears & Okamoto, 1958), hexaploid tall fescue (Jauhar 1975a,b), and hexaploid oat (Rajhathy & Thomas, 1972; Jauhar, 1977). The evolutionary and breeding implications of the genetic control of chromosome pairing were discussed in detail (Jauhar, 1975c). Such a genetic control brings about diploid-like chromosome pairing, which, in turn, ensures disomic inheritance. Thus, for alloploidy to become an important force in evolution and speciation, superimposition of a precise genetic control on chromosome pairing would be necessary. Jauhar (1975d) surmised, therefore, that 'polyploidy and sexuality cannot coexist in nature without such a regulatory mechanism.' Natural polyploids without a genetic control are mostly, if not exclusively, apomictic and hence blind alleys from the evolutionary stand point.

Even among the related sexual species, polyploidy exists in those that have developed a diploidizing mechanism. *Festuca* and its allied genus *Lolium* illustrate this point very well. The genus *Festuca* is characterized by the preponderance of polyploid taxa, most of them being allopolyploids ranging from $2n = 4x = 28$ to $2n = 10x = 70$ with genetically enforced diploid-like pairing (see Jauhar 1993, chapter 8). Thus, *Festuca* with the benefits of polyploidy and hybridity has become a large, diverse, and highly successful genus, well adapted to a wide variety of environments. In nature, polyploidy is in fact common in most genera of grasses. The genus *Lolium* is, however, an exception in this regard, all its species being diploids with $2n = 14$ chromosomes. Jauhar (1993) surmised that the absence of polyploidy in *Lolium* is due to the lack of a regulatory mechanism that controls chromosome pairing. Because species in this genus are closely related (Jauhar, 1975e), diploid-like pairing in their amphidiploids will probably not be achieved without a diploidizing mechanism of the type described in polyploid wheats and fescues.

Most, if not all, natural disomic polyploids have some form of control of chromosome pairing. Without such a control, precise bivalent formation in natural polyploids (with several sets of related chromosomes) would not be achieved. It is this fact that led Jauhar (1975d) to further modify the Blake-Haldane statement to read as: 'To create a little flower is the labour of ages except by allopolyploidy *coupled with genetic control of chromosome pairing*' (the italicized portion added by Jauhar). Thus three factors, viz., sexuality, allopolyploidy, and genetic control of chromosome pairing, together constitute a perfect recipe for rapid evolution and speciation in nature.

Conclusion and perspectives

Hybridization coupled with sexual polyploidization, via functioning of unreduced (2*n*) gametes in the parental species or in their hybrids, has been a major force in the evolution of our crop plants that sustain us. It has been speculated for a long time (see Harlan & de Wet, 1975) that 2*n* gametes have been largely instrumental in producing the widespread occurrence of polyploidy in nature. In this article and the earlier one (Jauhar et al., 2000), we present clear evidence of the functioning of 2*n* gametes in durum haploids leading to viable seed set and formation of disomic durum wheat. Using fl-GISH analysis of both somatic and meiotic chromosomes, we have demonstrated the precise duplication of the A- and B-genome chromosomes. We have presented strong evidence to show that the *Ph1*-induced failure of pairing in haploids may be a prerequisite for the occurrence of meiotic restitution and hence, chromosome doubling. The evolutionary and breeding implications of this phenomenon are discussed. It is also emphasized that a combination of the three factors namely, sexuality, allopolyploidy, and genetic control of chromosome pairing, constitutes a perfect recipe for rapid or cataclysmic evolution in nature.

References

Adams, W.T. & R.W. Allard, 1977. Effect of polyploidy on phosphoglucose isomerase diversity in *Festuca microstachys*. Proc Natl Acad Sci USA 74: 1652–1656.

Almouslem, A.B., P.P. Jauhar, T.S. Peterson, V.R. Bommineni & M.B Rao, 1998. Haploid durum wheat production via hybridization with maize. Crop Sci 38: 1080–1087.

Baenziger, P.S., 1996. Reflections on doubled haploid in plant breeding: *In Vitro* Haploid Production in Higher Plants. Volume 1: Fundamental Aspects and Methods, pp 35–48. Kluwer Academic Publishers, Dordrecht, Boston & London.

Blake, W., 1792. The Marriage of Heaven and Hell. (privately printed)

Blakeslee, A.F. & J. Belling, 1924. Chromosomal mutations in the Jimson weed, *Datura stramonium.* J Hered 15: 195–206.

Buso, J.A., L.S. Boiteux, G.C.C. Tai & S.J. Peloquin, 1999. Chromosome regions between centromeres and proximal crossovers are the physical sites of major effect loci for yield in potato: Genetic analysis employing meiotic mutants. Proc Natl Acad Sci USA 96: 1773–1778.

Carnahan, H.L. & H.D. Hill, 1961. Cytology and genetics of forage grasses. Bot Rev 27: 1–162.

Cox, T.S., 1998. Deepening the wheat gene pool. In: A.S. Basra (Ed.), Crop Sciences: Recent Advances. J Crop Prod 1: pp. 1–25. The Haworth Press Inc., New York.

Crowley, J.G. & H. Rees, 1968. Fertility and selection in tetraploid *Lolium.* Chromosoma 24: 300–308.

Curtis, C.A. & G.G. Doyle, 1992. Production of aneuploid and diploid eggs by meiotic mutants of maize. J Hered 83: 335–341.

D'Amato, F., 1990. Somatic nuclear mutations *in vivo* and *in vitro* in higher plants. Caryologia 43: 191–204.

de Wet, J.M.J., 1980. Origins of polyploids. In: W.H. Lewis (Ed.), Polyploidy: Biological Relevance, pp. 3–15. Plenum Press, New York and London.

Doğramacı-Altuntepe, M. & P.P. Jauhar, 2001. Production of durum wheat substitution haploids from durum × maize crosses and their cytological characterization. Genome 44: 137–142.

Doğramacı-Altuntepe, M., T.S. Peterson & P.P. Jauhar, 2001. Anther culture-derived regenerants of durum wheat and their cytological characterization. J Hered 92: 56–64.

Dvořák, J., 1998. Genome analysis in the *Triticum-Aegilops* alliance. Proc 9th Int Wheat Genet Symp, University of Saskatchewan, Saskatoon, Canada 1: pp. 8–11.

Dvořák, J., P. DiTerlizzi, H.-B. Zhang & P. Resta, 1993. The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21–31.

Dvořák, J., M.-C. Luo, Z.-L. Yang & H.-B. Zhang, 1998. The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97: 657–670.

Ghaemi, M., A. Sarrafi, H. Barthou & G. Alibert, 1993. Genetic variability for anther culture responses in tetraploid and hexaploid wheat species. Cereal Res Commun 21: 231–237.

Giorgi, B., 1978. A homoeologous pairing mutant isolated in *Triticum durum* cv. Cappelli. Mutation Breed Newsl 11: 4–5.

Grant, V., 1963. The Origin of Adaptations. Columbia Univ. Press, New York.

Grant, V., 1981. Plant Speciation. 2nd edn. Columbia Univ Press, New York.

Grimanelli, D., O. Leblanc, E. Espinosa, E. Perotti, D. Gonzalez de Leon & Y. Savidan, 1998. Non-mendelian transmission of apomixis in maize-*Tripsacum* hybrids caused by a transmission ratio distortion. Heredity 80: 40–47.

Harlan, J.R. & J.M.J. de Wet, 1975. On Ö. Winge and a prayer: The origins of polyploidy. Bot Rev 41: 361–390.

Haldane, J.B.S., 1958. The theory of evolution, before and after Bateson. J Genet 56: 1–17.

Heyn, F.W., 1977. Analysis of unreduced gametes in *Brassiceae* by crosses between species and ploidy levels. Z Pflanzenzüchtg 78: 13–30.

Huang, S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill, R. Haselkorn & P. Gornicki, 2002. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Trit-*

icum/Aegilops complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci USA 99: 8133–8138.

Hutchinson, G.E., 1953. Marginalia. Amer Sci 41: 630.

Iwanaga, M. & S.J. Peloquin, 1979. Synaptic mutant affecting only megasporogenesis in potatoes. J Hered 70: 385–389.

Jain, S.M., S.K. Sopory & R.E. Veilleux, 1996. *In Vitro* Haploid Production in Higher Plants, volume 1: Fundamental Aspects and Methods. Kluwer Academic Publishers, Dordrecht, Boston & London.

Jauhar, P.P., 1970. Chromosome behaviour and fertility of the raw and evolved synthetic tetraploids of pearl millet, *Pennisetum typhoides* Stapf et Hubb. Genetica 41: 407–424.

Jauhar, P.P., 1975a. Genetic control of diploid-like meiosis in hexaploid tall fescue. Nature 254: 595–597.

Jauhar, P.P., 1975b. Genetic regulation of diploid-like chromosome pairing in the hexaploid species, *Festuca arundinacea* Schreb. and *F. rubra* L. (Gramineae). Chromosoma 52: 363–382.

Jauhar, P.P., 1975c. Genetic control of chromosome pairing in polyploid fescues: Its phylogenetic and breeding implications. Rept Welsh Plant Breed Stn for 1974: 114–127.

Jauhar, P.P., 1975d. Polyploidy, genetic control of chromosome pairing and evolution in the *Festuca-Lolium* complex. Proc 178th Meeting of the British Genetical Society. John Innes Institute, Norwich, England, April 1975. Heredity 35: pp. 430.

Jauhar, P.P., 1975e. Chromosome relationships between *Lolium* and *Festuca* (Gramineae). Chromosoma 52: 103–121.

Jauhar, P.P., 1977. Genetic regulation of diploid-like chromosome pairing in *Avena*. Theor Appl Genet 49: 287–295.

Jauhar, P.P., 1981. Cytogenetics and Breeding of Pearl Millet and Related Species. Alan R. Liss., Inc., New York, 310 pp.

Jauhar, P.P., 1993. Cytogenetics of the *Festuca-Lolium* Complex: Relevance to Breeding. Springer-Verlag, Berlin, Heidelberg, Germany, 260 pp.

Jauhar, P.P., 1996. Genome analysis: a prologue. In: P.P. Jauhar (Ed.), Methods of Genome Analysis in Plants, pp. 1–6. CRC Press, Boca Raton.

Jauhar, P.P., 2003. Haploid and doubled haploid production in durum wheat by wide hybridization. In: M. Maluszynski, K.J. Kasha, B.P. Forster & I. Szarejko (Eds.), Doubled Haploid Production in Crop Plants: A Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands (in press).

Jauhar, P.P. & U. Singh, 1969: Amphidiploidization induced by decapitation in an interspecific hybrid of *Pennisetum*. Curr Sci 38: 420–421.

Jauhar, P.P., O. Riera-Lizarazu, W.G. Dewey, B.S. Gill, C.F. Crane & J.H. Bennett, 1991. Chromosome pairing relationships among the A, B, and D genomes of bread wheat. Theor Appl Genet 82: 441–449.

Jauhar, P.P., A.B. Almouslem, T.S. Peterson & L.R. Joppa, 1999. Inter- and intragenomic chromosome pairing relationships in synthetic haploids of durum wheat. J Hered 90: 437–445.

Jauhar, P.P., M. Doğramacı-Altuntepe, T.S. Peterson & A.B. Almouslem, 2000. Seedset on synthetic haploids of durum wheat: Cytological and molecular investigations. Crop Sci 40: 1742–1749.

Knox, R.E., J.M. Clarke & R.M. DePauw, 2000. Dicamba and growth condition effects on doubled haploid production in durum wheat crossed with maize. Plant Breed 119: 289–298.

Lassner, M.W. & T.J. Orton, 1983. Detection of somatic variation. In: S.D. Tanksley & T.J. Orton (Eds.), Isozymes in Plant Genetics and Breeding, Part A. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Lewis, H., 1966. Speciation in flowering plants. Science 152: 167–172.

Lewis, W.H. (Ed.), 1980. Polyploidy: Biological Relavance. Plenum Press, New York and London.

Maan, S.S. & T. Sasakuma, 1977. Fertility of amphihaploids in Triticinae. J Hered 68: 87–94.

McFadden, E.S. & E.R. Sears, 1946. The origin of *Triticum spelta* and its free threshing hexaploid relatives. J Hered 37: 81–89.

Mok, D.W.S. & S.J. Peloquin, 1975. Three mechanisms of 2*n* pollen formation in diploid potatoes. Can J Genet Cytol 17: 217–225.

Nishikawa, K., 1983. Species relationship of wheat and its putative ancestors as viewed from isozyme variation. Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp. 59–63.

Nygren, A., 1954. Apomixis in the angiosperms. Bot Rev 20: 577–649.

O'Donoughue, L.S. & M.D. Bennett, 1994. Comparative responses of tetraploid wheats pollinated with *Zea mays* L. and *Hordeum bulbosum* L. Theor Appl Genet 87: 673–680.

Okwuagwu, C.O. & S.J. Peloquin, 1981. A method of transferring the intact parental genotype to the offspring via meiotic mutants. Am Potato J 58: 512–513.

Otto, S.P. & T. Lenormand, 2002. Resolving the paradox of sex and recombination. Nature Rev Genet 3: 252–262.

Peloquin, S.J., 1982. Meiotic mutants in potato breeding. Stadler Symp 14: 99–109.

Phillips, R.L., 1981. Plant cytogenetics. In: G. Clark (Ed.), Staining Procedures, 4th edn., pp. 341–359. Williams & Wilkins, Baltimore, London, Los Angeles, Sydney.

Rajhathy, T. & H. Thomas, 1972. Genetic control of chromosome pairing in hexaploid oats. Nature New Biol 239: 217–219.

Ramanna, M.S., 1974. The origin of unreduced microspores due to aberrant cytokinesis in the meiocytes of potato and its genetic significance. Euphytica 23: 20–30.

Ramanna, M.S., 1979. A reexamination of the mechanism of 2n gamete formation in potato and its implication for breeding. Euphytica 28: 537–561.

Ramanna, M.S., 1983. First division restitution gametes through fertile desynaptic mutants of potato. Euphytica 32: 337–350.

Rice, W.R., 2002. Experimental tests of the adaptive significance of sexual reproduction. Nature Rev Genet 3: 241–251.

Riley, R. & V. Chapman, 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182: 713–715.

Sarrafi, A., N. Amrani & G. Alibert, 1994. Haploid regeneration from tetraploid wheat using maize pollen. Genome 37: 176–178.

Sears, E.R. & M. Okamoto, 1958. Intergenomic chromosome relationships in hexaploid wheat. Proc X Intern Congr Genet 2: 258–259.

Sibi, M.L., A. Kobaissi & A. Shekafandeh, 2001. Green haploid plants from unpollinated ovary culture in tetraploid wheat (*Triticum durum* Desf.). Euphytica 122: 351–359.

Stebbins, G.L., 1956. Cytogenetics, evolution and plant breeding. Indian J Genet Pl Breed 17: 129–141.

Stebbins, G.L., 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18: 26–35.

Stebbins, G.L., 1974. Flowering Plants: Evolution Above the Species Level. The Belknap Press of Harvard University Press. Cambridge, Massachusetts.

Stefani, A., 1986. Unreduced gametes in the F_1 hybrid of *Triticum durum* Desf. × *Haynaldia villosa* Schur. Z Pflanzenzüchtg 96: 8–14.

Stelly, D.M. & S.J. Peloquin, 1986. Formation of 2n megagametophytes in diploid tuber-bearing *Solanums*. Am J Bot 73: 1351–1363.

Vig, B.K., 1973. Somatic crossing over in *Glycine max* (L.) Merrill: Effect of some inhibitors of DNA synthesis on the induction of

somatic crossing over and point mutations. Genetics 73: 583–596.

Wang, G.Z., N.T. Miyashita & K. Tsunewaki, 1997. Plasmon analyses of *Triticum* (wheat) and *Aegilops*: PCR single-strand conformational polymorphism (PRC-SSCP) analyses of organellar DNAs. Proc Natl Acad Sci USA 94: 14570–14577.

Watanabe, K. & S.J. Peloquin, 1989. Occurrence of 2n pollen and *ps* gene frequencies in cultivated groups and their related wild species in tuber-bearing *Solanums*. Theor Appl Genet 78: 329–336.

Xu, S.J. & L.R. Joppa, 1995. Mechanisms and inheritance of first division restitution in hybrids of wheat, rye and *Aegilops squarrosa*. Genome 38: 607–615.

Xu, S.J. & L.R. Joppa, 2000. First-division restitution in hybrids of Langdon durum disomic substitution lines with rye and *Aegilops squarrosa*. Plant Breed 119: 233–241.